Institute Report No. 263

Dermal Sensitization Potential of Nitroguanidine in Guinea Pigs

Earl W. Morgan, MAJ, VC
Gerald F.S. Hiatt, PhD
and
Don W. Korte, Jr, PhD, MAJ, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

March 1988

Toxicology Series: 117

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 263	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Mammalian Toxicology Branch, Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-UL-TO-M	7a. NAME OF MONITORING ORGANIZATION: US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62720A; PROJECT NO. 835; TASK NO. A5; WORK UNIT ACCESSION NO. DA 303913

11. TITLE (Include Security Classification): Dermal sensitization potential of nitroguanidine in guinea pigs

12. PERSONAL AUTHOR(S): Earl W. Morgan, MAJ, VC; Gerald F.S. Hiatt, PhD; and Don W. Korte, Jr., PhD, MAJ, MS

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 22 Aug 84 TO 12 Oct 84	March 1988	25

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): dermal sensitization, toxicology, nitroguanidine, Buehler test, guinea pigs

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Nitroguanidine was tested for its potential to produce sensitization via contact with the skin. Testing on male guinea pigs was performed using the Buehler Dermal Sensitization Method. No evidence of dermal sensitization to nitroguanidine was obtained in this study.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Edwin S. Beatrice, COL, MC	22b TELEPHONE (Include Area Code): (415) 561-3600; 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

ABSTRACT

Nitroguanidine was tested for its potential to produce sensitization via contact with the skin. Testing on male guinea pigs was performed using the Buehler Dermal Sensitization Method. No evidence of dermal sensitization to nitroguanidine was obtained in this study.

Key Words: Dermal Sensitization, Toxicology, Nitroguanidine, Buehler Test, Guinea Pigs

PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT/APC: 180 Environmental Health Effects of Army Materials/TLB0

GLP STUDY NO.: 84027

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ, MSC

PRINCIPAL INVESTIGATOR: Earl W. Morgan, MAJ, VC, Diplomate, American College of Veterinary Preventive Medicine and American Board of Toxicology

CO-PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 22 Aug - 12 Oct 1984

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of nitroguanidine in guinea pigs.

ACKNOWLEDGMENTS

LTC Larry D. Brown, DVM, and SP4 Steven K. Sano, BS, provided research assistance; Yvonne C. Johnson, BS, provided statistical and research assistance; Richard D. Spieler, Richard Katona, and Charlotte L. Speckman provided animal care and facility management; Callie B. Crosby, MA, and Brenda Goce provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84027 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 11 July 85

DON W. KORTE JR., PhD / DATE
MAJ, MS
Study Director

Earl W. Morgan 19 June 85

EARL W. MORGAN, DVM / DATE
CPT, VC
Principal Investigator

Gerald F.S. Hiatt 21 June 85

GERALD F.S. HIATT, PhD / DATE
DAC
Co-Principal Investigator

Conrad Wheeler 21 June 85

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 22 March 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for GLP Study 84027

1. I hereby certify that in relation to LAIR GLP Study 84027, Tox Series 117, the following inspections were made:

27 July 1984	-	Protocol Review
20 September 1984	-	Dosing

2. The report and raw data were reviewed on 12 December 1986.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Dermal Sensitization Potential of Nitroguanidine in Guinea Pigs--Morgan *et al*

INTRODUCTION

Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Toxicology Branch, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of nitroguanidine in guinea pigs.

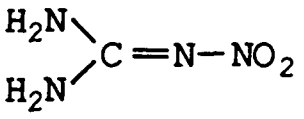
MATERIALS

Test Substance

Chemical name: Nitroguanidine

Chemical Abstract Service Registry No.: 556-88-7

Structural formula:



Molecular formula: $CH_4N_4O_2$

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

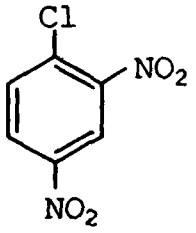
Sterile isotonic saline (Travenol Laboratories, Deerfield, IL) was used as the vehicle for nitroguanidine. The expiration date for this lot (8C865A4) was December 1984.

Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)

Chemical Abstract Service Registry No.: 97-00-7

Structural formula:



Molecular formula: $C_6H_3N_2O_4Cl$

Vehicle for Positive Control

The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc, (Philadelphia, PA). The same lot of saline was used as for the nitroguanidine vehicle. Other positive control substance information is presented in Appendix A.

Animal Data

Forty-seven male guinea pigs, Hartley strain (Simonsen Laboratories, Gilroy, CA), were used in the study. They were identified individually with ear tags numbered 84E0093 to 84E0139. At time of receipt two animals were selected, at random, for quality control necropsy evaluation. A pilot study to determine a non-irritating dose level used four animals. One was removed from the study because of broken leg, one broke his leg and was removed during quarantine, and one died during quarantine. Animal weights on receipt ranged from 140 to 233 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow Diet 5026 (Ralston Purina Company, Checkerboard Square, St Louis, MO 63188); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 22.2°C to 25.6°C and relative humidity in a range of 28 to 50%, with occasional spikes as high as 70% (room washing). The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (1) and EPA guidelines (2).

Group Assignment/Acclimation

The guinea pigs were quarantined for 21 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Ten animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

Dosage Levels

Nitroguanidine was applied as a 10% solution in isotonic saline. A pilot study, using 100%, 10%, 1%, and 0.1% concentrations, indicated the 10% solution to be the highest non-irritating concentration under the conditions of this test.

Two sensitization control groups were included in the study. Dinitrochlorobenzene, a known potent sensitizing agent (3), was applied to one group, at a 0.1% concentration, as a positive control. Isotonic saline was applied to another group as a vehicle control. In addition, a negative control group received nitroguanidine only on the day of challenge dosing.

Compound Preparation

The test compound was prepared by mixing 0.5 g nitroguanidine with 0.5 ml of isotonic saline to make a

paste. The dinitrochlorobenzene dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (4-6). Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, negative control groups were added which received only the challenge dose.

During the induction phase, the experimental, saline control, and positive control groups were dosed with 0.5 ml of the appropriate compound applied topically under a one-inch square gauze patch. This procedure was performed for three consecutive weeks (12 Sep, 19 Sep, and 26 Sep 84). The day before each dosing a three-inch square area on the left side of the animal was clipped with electric clippers (Oster™ Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI 53217) and then shaved with an electric razor (Norelco™ Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT 06904). The patch was taped with Blenderm™ hypo-allergenic surgical tape (3M Corp., St. Paul, MN 55144) to the same site each time and the animal was wrapped several times with Vetrap™ (same source). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was marked off for scoring.

Animals were challenged two weeks (10 Oct 84) following the third induction dose. The experimental group and the positive control group received two 0.5 ml doses, one applied to the old site on the left side and the other to a new site on the right side. Negative and vehicle control groups only received a single 0.5 ml dose which was applied to the left side. The procedures for clipping, shaving, wrapping, and exposure period remained the same.

In Buehler's procedure (4-6), skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24 and 48 hours after each induction dose as well. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema) and 3 (marked erythema). The results are expressed both in terms of incidence (the number of animals showing responses of 1 or greater at either 24 or 48 hours) and severity (the sum of the test scores divided by the number of animals tested). Results from the left side are compared with right side and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (6) also recommended depilating the day before the challenge dose is applied. For consistency with induction procedures, this step was replaced by clipping the animals as described previously.

A historical listing of study events is provided in Appendix C.

Deviations from Study Protocol

A 0.5 level (very slight erythema) was added to the scoring system to allow for borderline responses.

The DNCB solution was maintained at approximately 65°C before dosing the guinea pigs. This was necessary to keep the DNCB in solution, but did not result in thermal insult to the animals' skin as the aliquot for dosing cooled quickly during pipetting and application to the patch. Significant sensitization was produced by DNCB with this method.

The guinea pigs received were younger and thus smaller than requested. The quarantine period was extended one week to allow them to grow to the desired size. However, due to their smaller size, two of the animals got their legs caught in the wire mesh bottoms of the cages. They sustained broken legs and were terminated. Since a reduced number of guinea pigs was available, two groups had to be reduced to 9 animals each.

It is believed that these deviations from the protocol did not adversely affect study results.

RESULTS

Tables 1 and 2 summarize the incidence of reactions 24 and 48 hours after each dose. There were no reactions observed in response to nitroguanidine administration, either at 24 or 48 hours.

TABLE 1

Incidences of Skin Reactions after 24 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Nitroguanidine	0/10	0/9	0/9	0/9	0/9
Negative Control*	----	---	---	0/9	0/9
Saline Vehicle	1/10	0/10	0/10	1/10	0/10
DNCB	1/10	4/10	4/10	9/10	8/10

* The Negative Control Group received only a challenge dose of the test compound.

TABLE 2

Incidences of Skin Reactions after 48 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Nitroguanidine	0/10	0/9	0/9	0/9	0/9
Negative Control*	----	---	---	0/9	0/9
Saline Vehicle	0/10	0/10	0/10	0/10	0/10
DNCB	1/10	2/10	6/10	9/10	6/10

*The Negative Control Group received only a challenge dose of the test compound.

This lack of response is reflected in Tables 3 and 4, which report the severity of skin reactions at 24 and 48 hours. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. This produced a severity index of 0.0 for nitroguanidine.

TABLE 3

Severity of Skin Reactions after 24 Hours

	Induction			Challenge	
Test Group	First	Second	Third	Left	Right
Nitroguanidine	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	0.0
Saline Vehicle	0.1	0.0	0.0	0.05	0.0
DNCB	0.05	0.3	0.2	1.0	0.95

* The Negative Control Group received only a challenge dose of the test compound.

TABLE 4

Severity of Skin Reactions after 48 Hours

	Induction			Challenge	
Test Group	First	Second	Third	Left	Right
Nitroguanidine	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	0.0
Saline Vehicle	0.0	0.0	0.0	0.0	0.0
DNCB	0.05	0.1	0.4	0.95	0.75

* The Negative Control Group received only a challenge dose of the test compound.

In contrast, dinitrochlorobenzene (DNCB) produced a positive response at all time points. Between 40% and 100% of the DNCB-treated animals exhibited a response 24 hours following the second and/or third induction and challenge doses. These reactions persisted, yielding scorable effects in 20 to 90% of the animals at 48 hours after dosing.

Severity scores for these responses to DNCB ranged from 0.2 to 1.0 at the 24-hour scoring period (Table 3). The highest score, 1.0, was observed on the left (induction) side in response to the challenge dose. By 48 hours the reactions had subsided somewhat; consequently, the severity range decreased to 0.1 to 0.95 (Table 4).

No responses whatsoever were observed in the negative control (challenge dose of nitroguanidine only) group. One animal in the vehicle control (saline-treated) group exhibited a positive response at the 24-hour scoring period for the first induction and the challenge dose. However, both times the dermal response on the animal had cleared by the 48-hour scoring period. The individual 24-hour and 48-hour scores for all animals appear, by group, in Appendix D.

DISCUSSION

Nitroguanidine was evaluated for its ability to elicit a delayed-hypersensitivity reaction via dermal contact. Using the method of Buehler and Griffith (4-6), we observed no response indicative of dermal sensitization produced by nitroguanidine. Therefore, in this study, nitroguanidine showed no evidence of potential to elicit an allergic response.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than man our results do not guarantee that nitroguanidine will not sensitize humans. The incidence data do indicate that nitroguanidine is <u>unlikely</u> to sensitize humans and that its potential is sufficiently low to permit testing in humans.

Any sensitization produced by nitroguanidine would have been easily detected by this study. An allergic response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously within the Institute (7). Although DNCB is capable of producing primary irritation, the responses observed in this study were indicative of an allergic reaction since the concentration of DNCB used for the induction and challenge doses was too low to produce primary

irritation. With the exception of a very slight irritation response in one animal 24 hours after the first induction, responses to DNCB were observed only after two or more exposures and the severity generally increased with the number of previous exposures.

CONCLUSIONS

Nitroguanidine, based on a zero percent sensitization rate in this study, exhibited no potential for inducing dermal sensitization.

REFERENCES

1. Buehler dermal sensitization test. LAIR Standard Operating Procedure OP-STX-82, Letterman Army Institute of Research, Presidio of San Francisco, CA. 18 May 1984.

2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Dermal sensitization. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Landsteiner K, Jacobs J. Studies on sensitization of animals with simple chemical compounds. J Exp Med 1935; 61:643-656.

4. Buehler EV. Delayed contact hypersensitivity in the guinea pig. Arch Dermatol 1965; 91:171-175.

5. Griffith JF. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Pharmacol 1969; Suppl 3:90-102.

6. Buehler EV, Griffith JF. Experimental skin sensitization in the guinea pig and man. In: Maibach HI, ed. Animal models in dermatology. Edinburgh: Churchill Livingstone 1975:56-66.

7. Lewis CM, Johnson YC, Korte DW. Dermal sensitization potential of the Holston compounds: virgin DMSO, DMSO recycle solvent, and DMSO evaporator sludge. Toxicology Series 70. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1984. Institute Report No. 172.

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Appendix A: CHEMICAL DATA

Chemical Name: Nitroguanidine (NGu)

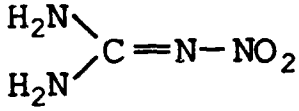
Other Listed Names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

Lot Number: SOW83H001-004

LAIR Code: TP36

Chemical Structure:



Molecular Formula: $CH_4N_4O_2$

Molecular Weight: 104.1

Physical State: White powder

Melting Point: 232° C[1]

Names of Contaminants and Percentages: (Data Sheet Attached)

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Analytical Data:
An infrared spectrum was obtained upon receipt of the compound; major absorption peaks were observed at 3330 (broad), 1660, 1630, 1525, 1400, 1300, 1050, and 780 cm^{-1}.[2] The spectrum was identical to the Sadtler spectrum for nitroguanidine.[3]

---

[1]Fedoroff BT, Sheffield OE. Encyclopedia of explosives and related items. Vol 6. Dover, New Jersey: Picatinny Arsenal, 1975: G154.

[2]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 39. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #21421.

Appendix A (cont.): CHEMICAL DATA

Stability:

An aqueous solution of NGu (48.1 µmolar) was prepared and the absorption at 264 nm determined to be 0.689 AUFS. Three weeks later the same solution was reexamined spectroscopically and the absorption at 264 nm found to be 0.689 AUFS. A full spectrum scan revealed the characteristic pattern of absorption in the UV range with peak maxima at 215 and 264 nm. These data indicate that NGu is stable in aqueous solution for at least three weeks.[4]

---

[4]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, pp 22 and 36. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): Nitroguanidine

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC
(DRSAR-P-702-109)

RCS CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE: 1 OF 1

TO: Commander, US Army Armament Munitions and Chemical Command, Attn: DRSMC-MAD, Rock Island, ILL. 61201

FROM: Sunflower Army Ammunition Plant, DeSoto, Kansas 66018

DATE: September 13, 1983

MATERIAL: Nitroguanidine Type II, Class 2 *

MANUFACTURER: Hercules Aerospace Division, Hercules Incorporated

CONTRACT NO.: DAAA-09-77-C-4016, CLIN 0270

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
SOW83H001-004		1	7,000 lbs.

PLACE MANUFACTURED: Sunflower Army Ammunition Plant, DP Facility

SPECIFICATION AND AMENDMENT/DRAWING NO.: MIL-N-494A w/Int. Amend 6 (AR) dated 25 March 1981 *

SECTION B - DESCRIPTION OF MATERIAL

Property	Requirement Min.	Requirement Max.	Analysis
Purity, %	99.0		99.6
Ash Content, %		0.30	0.03
pH Value	4.5	7.0	7.55 **
Acidity (as H_2SO_4), %		0.06	ND ***
Total Volatiles, %		0.25	0.03
Sulfates (as $NaSO_4$), %		0.20	0.01
Impurities, H_2O Insoluble, %		0.20	0.01
Particle Size, Microns		3.0 *	4.0 ****
Particle Size, Std. Dev.		± 0.5	0.168

* As amended by Contract Scope of Work
** Approved by Waiver No. NQ83-1 dated Sept. 2, 1983
*** ND = None Detected
**** Approved by Waiver No. NQ83-2 dated Sept. 9, 1983

REMARKS
1.) Manufactured under SOW ES 1A-3-8423, Nitroguanidine Particle Size, dated 1 Feb. 83.

2.) Packaging: Level B - fiber drums to Spec. DOT 21C60. Drums numbered 3 thru 243 and 247 thru 285. 25 pounds per drum per HAD letter dated Aguust 1, 1983, to COR.

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: Hercules Aerospace Division

TESTING CONDUCTED BY: Hercules Aerospace Division

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

13 Sept '83 — A. W. English (DATE — A. W. ENGLISH SIGNATURE)

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

14 Sep 83 (DATE) — Quality Assurance Specialist (TITLE)

FOR THE COMMANDER

M. A. Kozak (SIGNATURE)

ARRCOM FORM 213-R, 10 AUG 77 — REPLACES ARMCOM FORM 213-R, 15 JUL 75 (TEMP) WHICH MAY BE USED.

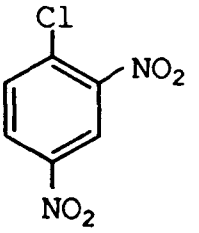
Appendix A (cont.): Positive Control

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52-54° C[1]

Purity:

The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[2] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (δ).[3] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[4]

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[5] Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ($CDCl_3$): δ 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[6] GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces

Appendix A (cont.): Positive Control

of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[7]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

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[1]Windholz M, ed. The Merck Index. 5th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

[2]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]*Ibid.* pp 11-12.

[4]*Ibid.* pp 13-16.

[5]Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[6]Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[7]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: Cavia porcellus

Strain: Hartley

Source: Simonsen Laboratories
Gilroy, CA

Sex: Male

Date of birth: 12 August 1984

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tagging procedure, tag numbers 84E0093 to 84E0139 inclusive.

Pretest conditioning: Quarantine/acclimation 22 Aug - 11 Sep 84

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF EVENTS

Date	Event
22 Aug 84	Forty-seven animals arrived, were examined, placed in cages, and fed.
23 Aug 84	Animals ear-tagged and weighed. Two animals submitted for necropsy as quality controls.
22 Aug, 12 Oct 84	Animals checked daily.
23, 28 Aug 4, 11, 18, 25 Sep 2, 9 Oct 84	Animals weighed.
29 Aug 84	Sacrificed 1 animal due to a broken leg.
4 Sep 84	Four pilot animals randomly selected and shaved. Pilot dosing solution prepared. One animal (84E095) found dead.
5 Sep 84	Pilot animals patch tested.
6 Sep 84	Pilot animals scored for 24-hour skin reaction.
7 Sep 84	Pilot animals scored for 48-hour skin reaction.
10 Sep 84	Pilot results evaluated, test concentration determined.
11 Sep 84	Animals randomized into groups.
11, 18, 25 Sep 84	Test animals, except negative control group, clipped and shaved. Dosing solutions prepared.

Appendix C (cont.): HISTORICAL LISTING OF EVENTS

Date	Event
12,19,26 Sep 84	Test animals, except negative control group, given induction dose.
13, 20, 27 Sep 84	Test animals, except negative control group, scored for 24-hour skin reaction.
14,21,28 Sep 84	Test animals, except negative control group, scored for 48-hour skin reaction.
14 Sep 84	Sacrificed 1 animal due to a broken leg.
9 Oct 84	Test animals clipped and shaved. Dosing solutions prepared.
10 Oct 84	Test animals given challenge dose.
11 Oct 84	Test animals scored for 24-hour skin reaction.
12 Oct 84	Test animals scored for 48-hour skin reaction. Thirty-eight animals sacrificed by CO asphyxiation.

Appendix D: INDIVIDUAL DERMAL SCORES

Page

Appendix D-1: Nitroguanidine

Severity of Skin Reactions 24 and 48 Hours After Compound Administration

Animal (84E0---)	INDUCTION DOSES						CHALLENGE DOSE			
	FIRST		SECOND		THIRD		LEFT FLANK		RIGHT FLANK	
	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H
104	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
105	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
107	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
117	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
121	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
127	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
134	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
135	0.0	0.0								
136	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
137	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix D-2: Dinitrochlorobenzene

Severity of Skin Reactions 24 and 48 Hours After Compound Administration

Animal (84E0---)	INDUCTION DOSES						CHALLENGE DOSE			
	FIRST		SECOND		THIRD		LEFT FLANK		RIGHT FLANK	
	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H
106	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
109	0.0	0.0	0.5	0.0	0.0	1.0	1.0	1.0	0.5	1.0
111	0.5	0.5	0.0	0.0	0.5	0.5	1.0	1.0	0.0	0.0
113	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
115	0.0	0.0	0.0	0.0	0.5	0.5	1.0	0.5	1.0	0.0
125	0.0	0.0	0.5	0.0	0.0	0.5	0.5	0.5	1.0	1.0
126	0.0	0.0	0.0	0.0	0.5	1.0	1.0	1.0	1.0	0.5
128	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
131	0.0	0.0	0.0	0.5	0.0	0.5	2.0	2.0	2.0	2.0
139	0.0	0.0	1.0	0.5	0.5	0.0	2.0	2.0	2.0	2.0
AVERAGE	0.05	0.05	0.3	0.1	0.2	0.4	1.0	0.95	0.95	0.75

Appendix D-3: Saline

Severity of Skin Reactions 24 and 48 Hours After Compound Administration

Animal (84E0---)	INDUCTION DOSES						CHALLENGE DOSE			
	FIRST		SECOND		THIRD		LEFT FLANK		RIGHT FLANK	
	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H
097	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
103	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
114	1.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
123	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
124	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
129	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
130	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
132	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
138	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.1	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0

Appendix D-4: Negative Control

Severity of Skin Reactions 24 and 48 Hours After Compound Administration

Animal (84E0---)	INDUCTION DOSES						CHALLENGE DOSE			
	FIRST		SECOND		THIRD		LEFT FLANK		RIGHT FLANK	
	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H
101							0.0	0.0	0.0	0.0
102							0.0	0.0	0.0	0.0
108							0.0	0.0	0.0	0.0
110							0.0	0.0	0.0	0.0
112							0.0	0.0	0.0	0.0
116							0.0	0.0	0.0	0.0
118							0.0	0.0	0.0	0.0
120							0.0	0.0	0.0	0.0
133							0.0	0.0	0.0	0.0
AVERAGE							0.0	0.0	0.0	0.0

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314